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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 9788 for a patent by BIOGLOBAL PTY LTD as filed on 02 January 2002.



WITNESS my hand this
Twentieth day of January 2003

A handwritten signature in cursive script that reads "J. Yabsley".

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Invention Title: **"NOCTUID ATTRACTANT COMPOSITION"**

The invention is described in the following statement:

"NOCTUID ATTRACTANT COMPOSITION"

BACKGROUND OF THE INVENTION

Field of the Invention

5 This invention relates to a novel noctuid attractant composition and its use as an attractant for both male and female noctuids and other lepidopteran pests.

Description of the Prior Art

10 Phenylacetaldehyde has long been known as an attractant for many species of Lepidoptera and is known to be an attractive component in corn silks. (Cantelo.W.W. and Jacobson, M. Environmental Entomology 8, 444, 1979; Cantelo.W.W. and Jacobson, M. J. Environ. Sci. Health A14, 695, 1979; CRC Handbook of Natural Pesticides Volume V1 1990).

15 The United States Department of Agriculture conducted research on the attractancy of volatiles from flowers of the Japanese Honeysuckle. As a result US Patent 6,190,65 was granted which describes the use of cis-jasmone alone or in combination with other attractants including linalool and phenylacetaldehyde as an attractant for Lepidoptera.

20 In a more extensive study, the United States Department of Agriculture conducted experiments bioassaying a range of plant volatile compounds to determine their attractancy for *Helicoverpa zea*. These experiments were carried out primarily with olfactometers. This work led to the invention described in US Patent 6,074,634 Lopez, Jr. , et al. which was
25 granted on June 13, 2000. This reference described an attractant for a range of adult Lepidopteran species which consisted of at least phenylacetaldehyde, methyl salicylate and methyl 2-methoxybenzoate and preferably 2-phenethyl alcohol and limonene.

30 The work leading to the advent of the present invention was done in an attempt to improve the utility of blends developed by previous workers in respect of their utility for the control of *Helicoverpa armigera* , a key pest in Australia and not present in USA.

SUMMARY OF THE INVENTION

An attractant composition for adult noctuid or other lepidopteran species comprising a mixture of about 10-45% by weight phenylacetaldehyde, 10-30% by weight caryophyllene including beta caryophyllene, 10-30% by weight 4 methoxy 2-phenethyl alcohol and 5-30% by weight Z,3 hexenyl salicylate. There also may be provided 0-30% anethole, 0-30% 4-methoxy benzyl alcohol, 0-30% 2 methoxy benzyl alcohol, 0-30% methyl anisate and 0-30% methyl anthranilate.

The present invention also provides methods for a reduction in noctuid and/or other lepidopteran species numbers, which includes attracting the insect species with the attractant composition. In the preferred embodiment, the attractant composition is used to pull the adult noctuid and/or other lepidopteran insects from the surrounding areas. The attractant is combined with or used in the vicinity of the food source containing an insecticide. The responding adults feed and as a result are killed.

In an alternative embodiment, the insecticide may be replaced with a pathogen such as nuclear polyhedrosis virus. In this case, the moths are not killed but their bodies become heavily contaminated and serve to distribute the virus through the crop.

Another object of the invention is to provide traps and controlled-release formulations containing the attractant composition.

A further object of the invention is to provide a method of reducing or preventing plant damage due to noctuid and/or other lepidopteran species using the attractant composition in combination with a feeding stimulant and insecticide.

The invention has certain advantages over that described in US Patent 6074634 Lopez et al. Our tests showed the attractancy of our compositions was at least equal to that described in the US Patent 6074634 for *Helicoverpa armigera* but the compounds used were generally of a higher molecular weight and lower volatility. As a consequence, the attractant blend of the present invention has greater utility in that it is relatively easy to formulate in a manner that will release for a prolonged time. It has

advantages over the blend described in US Patent 6,190,65 in that the ingredients are less costly.

DETAILED DESCRIPTION OF THE INVENTION

5 The attractant compositions of the present invention are effective for attracting and controlling a variety of agronomically important adult insects of the order Lepidoptera. While pests of particular importance which are attracted to the compositions include noctuids such as *Helicoverpa armigera* (corn earworm, cotton bollworm), *Helicoverpa punctigera* (native budworm),
10 *Chrysodeixus argentifera* (loopers) and other lepidoptera such as *Leucania convecta* and *Spodoptera* spp.. Without being limited thereto, it is envisioned that the attractants of this invention may be used to attract and control many species of Lepidoptera.

 Furthermore, the attractant compositions of this invention are effective
15 for attracting both sexes of adult Lepidoptera. Since female moths are the reproductive sex capable of laying eggs, the capture of females could serve as a major tool in reducing succeeding populations.

 Suitable formulations may be prepared from these volatiles in isolated or impure form. However, as a practical matter, it is expected that
20 substantially pure volatiles will be formulated with an inert carrier for use as an insect attractant composition. The practitioner skilled in the art will also recognize that these volatiles may be formulated in liquid or solid form. Liquid carriers for use herein include but are not limited to water or organic solvents, such as polyols, esters and vegetable oils. Emulsions of waxes are
25 suitable and when combined with an emulsifier provide a simple controlled release mechanism for the volatile attractants. Suitable waxes include paraffin wax and beeswax but other waxes may be equally as effective. Suitable emulsifiers include but are not limited to sorbitan monostearate

 The attractant composition may be further formulated with a variety of
30 optional components or adjuvants, including but not limited to other plant volatiles, feeding stimulants such as sucrose or invert sugar and insect toxicants.

Yet other components which may be included in the formulation include humectants, preservatives, thickeners, antimicrobial agents, antioxidants, emulsifiers, film forming polymers and mixtures thereof.

5 Additives which retard or slow the volatilization of the active mixture are preferred. Humectants may include polyols, sugars and glycols. Antioxidants reduce polymerization of phenyl acetaldehyde are preferred. Film forming polymers include gum rosin, latex, polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl chloride, polyethylene, polyvinyl acetate and mixtures thereof. Additional optional additives include, shellac, methyl
10 methacrylate, and mixtures thereof.

In the preferred embodiment, feeding stimulants for the adult insects or moths are included in the attractant composition and function to induce the target insects to contact and/or ingest the bait. Without being limited thereto, feeding stimulants such as fructose, glucose, and particularly
15 sucrose, are preferred. It has been found that impure sugars such as molasses, raw cane sugar and invert raw sugar are less attractive than pure white cane sugar.

Another important component of the adult control system of the present invention is inclusion of insect toxicants or pesticides that are highly
20 toxic to the adult insects or moths, but do not significantly inhibit the attractance or feeding response when combined with a food source and applied to or in the vicinity (such as on plants or in a trap or bait station) of the crop plants treated with the feeding attractant.

Insect toxicants which may be included in the attractant composition
25 include but are not limited to insecticides such as carbaryl, methomyl, acephate, thiodicarb., cyfluthrin, malathion, chlorpyrifos, emamectin benzoate, abamectin, spinosad, endosulfan, and mixtures thereof. Bacterial and viral pathogens may also be included, as well as insect growth regulators or compounds eliciting behaviour modification or disrupting
30 physiological functions.

Combination of the insecticide with the attractant composition of this invention and concentrated sucrose allows the use of significantly lower

concentrations of insecticides to kill the adults under field conditions than would be used to control the insect pests with a normal commercial broadcast application of the same insecticides. Accordingly, one advantage of the present invention is a decrease in amount and concentration of insecticides required as compared with conventional insecticidal crop protection.

Use in this manner should prove useful in suppressing target species before they can inflict damage to agronomically important crops.

In another embodiment, the attractant composition may be included as a part of a trap or other solid support which may also contain, be a part of or be in close proximity to a feeding stimulant, and/or an insecticide, pesticide, or other mechanical (such as a "bug zapper"), toxic or biologically active agent to eliminate, reduce, or prevent reproduction of the target insect species.

It is envisioned that the attractants may be used in conjunction with any type of appropriate trap or attractant disseminator as known in the art. The attractant can be applied or disseminated using a variety of conventional techniques, such as in an exposed solution, impregnated into a wicking material or other substrate, or incorporated in a deodorant dispenser. Further, the components of the attractant may be combined in a single dispenser provided within a single trap, or provided separately in a plurality of dispensers, all within a single trap. The attractant composition can be applied to the device undiluted, or formulated in an inert carrier. Volatilisation can be controlled or retarded by inclusion of components as described above. Controlled, slow release over an extended period of time may also be effected by placement within vials covered with a permeable septum or cap, by encapsulation using conventional techniques, or absorption into a porous substrate.

One of ordinary skill will appreciate that the rate of release of the active ingredient mixture of the present invention may be varied by manipulation of the size of the reservoir and permeability of the matrix. The support or other delivery mechanisms of the present invention preferably

provides release or volatilisation of the active ingredient mixture of the invention for at least one week.

Application scenarios and methods of using the attractant composition of the present invention also include separate application of a feeding stimulant-insecticide mixture to plants by known methods with the placement of the attractant composition in a manner which will attract the noctuid and/or other lepidopteran species to the feeding stimulant-insecticide mixture. Placement may include location in a strip in the same field which is upwind of the strip of the feeding stimulant-insecticide mixture. The attractant composition of the present invention may be applied in or on granules, plastic dispensers or wicks, for example, and may be applied parallel to sprays of a feeding stimulant-insecticide mixture. Cross-wind application may offer greater control of the insect population because of an increase in the area with effective volatile concentrations, and the foraging and ovipositing behaviour in which the moths fly upwind within the plant canopy. Single point application of the attractant composition may also be used effectively, depending on the existing wind conditions. Plants which may be protected from insect pests include but are not limited to agronomically important crops such as cotton and vegetables, sorghum, field corn, seed corn, sweet corn, cole crops and tomatoes.

In the practice of any of the above-described embodiments, an attractant is used as a trap bait or is otherwise applied to the locus of or in the vicinity of infestation in an amount effective to attract the target insect. Factors such as population density, precipitation, temperature, wind velocity, and release rate will influence the actual number of insects trapped.

EXPERIMENTAL SECTION

A useful formulation of the attractant blend is as follows:

Attractant blend

Ingredient	Percentage w/w
Phenylacetaldehyde	16.7
4 Methoxy phenylethanol	16.7

Z,3 Hexenyl salicylate	16.7
Caryophyllene	16.7
Anethole	16.7
4 Methoxybenzyl alcohol	16.7
Total	100.0

The above attractant blend is mixed with other components as follows:

Ingredient	Purpose	Grams per kilogram
Wax	Carrier	100.0
Attractant	active ingredient	10.0
Vitamin E	Antioxidant	1.0
BHT	Antioxidant	1.0
Kemotan *	Emulsifier	22.0
Xanthan	Thickener	0.5
Sugar	feeding stimulant	400.0
Water	Extender	464.5
Total		1000.0

* sorbitan monostearate

5

The above formulation is combined with water on a 1:1 volume/volume basis and a suitable amount of insecticide is added. The resultant dilute mixture is applied as a coarse spray at the rate of 10 – 20 litres per kilometre of row to one or two rows per 100 metres, preferably in late afternoon.

10

Comparisons of a wide range of attractant blends using 'sticky board' traps.

Results of traps include the following:

15

Comparison of attractant blends using sticky traps

Table 1 Comparison of moth counts in sticky traps

Treatment	Mean (Moths/board)**	Standard Dev.	Replicates
No treatment	1.19 a	1.60	16
PAA	1.00 a	1.00	9
Lopez	5.75 c	3.51	16

Treatment	Mean (Moths/board)**	Standard Dev.	Replicates
D23	4.41 bc	3.87	17
D24	3.18 b	2.32	17

* Total of all Noctuidae ** numbers followed by the same number are not significantly different $P = 0.05$. Students t test.

Blend D23 and Lopez were comparable but the D 24 caught significantly fewer moths than Lopez. D24 had a significantly lower count indicating that anethole was an effective addition to the D23 blend. Phenylacetaldehyde was not significantly attractive as a sole treatment.

Ingredients	Milligrams of attractant per sticky board				
	PAA	Lopez	D23	D23	D24
Phenylacetaldehyde	3.12	3.12	3.12	3.12	3.12
Caryophyllene	0	0	3.12	3.12	3.12
4 methoxy phenylethanol	0	0	3.12	3.12	3.12
2 methoxy benzyl alcohol	0	0	3.12	3.12	3.12
4 methoxy benzyl alcohol	0	0	0	0	0
Z,3 hexenyl salicylate	0	0	3.12	3.12	3.12
Anethole	0	0	3.12	3.12	0
Methyl salicylate	0	3.12	0	0	0
Phenylethanol	0	3.12	0	0	0
Limonene	0	3.12	0	0	0
Methyl 2-methoxybenzoate	0	3.12	0	0	0

10 Results of an experiment in control of *Helicoverpa armigera*

Location:

A mung bean field on the property of Kingsley Chapman near Oakey on the Darling Downs in Queensland.

15

Materials and methods

Background

20 The attractant blend described above can be used in a sprayable

formulation to be used in pest control in the following ways:

- 1) to draw adult *Helicoverpa armigera* to trap crops (small attractive crops grown beside the commercial crop – trap crops are mown or sprayed with an insecticide to kill the insects)
- 2) to draw adult *Helicoverpa armigera* into source crops (crops grown as sources of beneficial insects – predators and parasites of *Helicoverpa armigera*)
- 3) used as an 'attract and kill' product in a tank mix combination with an insecticide to draw adult *Helicoverpa armigera* the border of a commercial crop to control adult moths immigrating from outside sources. This has the potential to reduce drastically (to 1-2%) the amount of insecticide used to control the pest.

The following simple sprayable formulation was used in a preliminary trial

Ingredient	Grams
Beeswax	100
Attractant	5
kemotan	20
Water	440
Sugar	440
Total	1005

100 grams of beeswax was melted with 20 grams of sorbitan monostearate (emulsifier) and mixed until homogenous. 5 grams of the attractant mix is added to the wax emulsifier mixture and mixed until homogenous. The water was then added to form a wax in water emulsion. Finally, the sugar was added and mixed vigorously in a blender to give a the final formulation.

The above formulation was prepared using the attractant mixture listed below.

Ingredient	% w/w
Phenylacetaldehyde	20.00
Caryophyllene	20.00
2-Methoxybenzyl alcohol	20.00
Z,3 Hexenyl salicylate	20.00
4-Methoxyphenyl ethyl alcohol	20.00

5 1 litre of water was added to an equal volume litres of the above liquid to thin the attractant mix.

6.25 grams of 80% Carbaryl was added to the mixture and mixed thoroughly.

10 A mixture identical to the above but lacking any volatile attractant (other than those inadvertently present in raw beeswax) served as a control.

One litre of the resultant mixtures were applied as a coarse spray onto 4 rows x 50 metres of Mung beans which were known to support a high level of infestation of *Helicoverpa armigera*.

15 Adult *Helicoverpa armigera* killed by the treatment were collected from the treated and adjoining rows were collected and recorded one hour and 20 hours after application.

Results

Table 1 *Helicoverpa armigera* killed using the attract and kill formulation

20

Time after application (hours)	<i>Helicoverpa armigera</i>					
	Treatment			Control		
	Males	Females	Total	Males	Females	Total
1	56	11	67	5	2	7
20	1505	536	2049	686	265	951

When the experimental site was visited, a large flock of crows was observed to rise from the treatment area. The birds then moved in smaller numbers to the control area. It is likely that a significant proportion of the

moths in both test and control areas were consumed by birds prior to the evaluation of the trial.

5 The trial site had a very high population of *Helicoverpa armigera* moths. A significant number would have encountered the feeding stimulant by random movement. For this reason, the number in the control would be expected to be high after 20 hours.

Despite the limitations of this test, the results strongly support the following:

- 10 1 A sugar based 'attract and kill' product in combination with a common insecticide is capable of killing large numbers of the target pest, *Helicoverpa armigera*
- 2 The presence of the novel attractants in the 'attract and kill' product is likely to significantly enhance the effectiveness of
- 15 the product.

Dated this Second Day of January 2002

20

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By their Patent Attorney
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